PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 October 2000 (20.10.00)	
International application No. PCT/CA00/00190	**Applicant's or agent's file reference** 1038-1021MIS
International filing date (day/month/year) 24 February 2000 (24.02.00)	**Priority date** (day/month/year) 24 February 1999 (24.02.99)
Applicant SIA, Charles, D., Y. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 September 2000 (20.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Manu Berrod

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

STEWART, Michael, I.
Sim & Mcburney
6th Floor, 330 University Avenue
Toronto, Ontario M5G 1R7
CANADA

Date of mailing (day/month/year) 27 July 2000 (27.07.00)	
Applicant's or agent's file reference 1038-1021MIS	**IMPORTANT NOTIFICATION**
International application No. PCT/CA00/00190	**International filing date** (day/month/year) 24 February 2000 (24.02.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address	State of Nationality	State of Residence
PARRINGTON, Mark 45 Martin Street Bradford, Ontario L3Z 1Z4 Canada	CA	CA
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
New applicant/inventor for the United States only.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Athina Nickitas-Etienne
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 003435249

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

STEWART, Michael, I.
Sim & Mcburney
6th Floor, 330 University Avenue
Toronto, Ontario M5G 1R7
CANADA

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
01 August 2001 (01.08.01)

Applicant's or agent's file reference
1038-1021MIS:sd

IMPORTANT NOTIFICATION

International application No.
PCT/CA00/00190

International filing date (day/month/year)
24 February 2000 (24.02.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CONNAUGHT LABORATORIES LIMITED
1755 Steeles Avenue West
Toronto, Ontario M2R 3T4
Canada

State of Nationality: CA
State of Residence: CA
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
AVENTIS PASTEUR LIMITED
1755 Steeles Avenue West
Toronto, Ontario M2R 3T4
Canada

State of Nationality:
State of Residence:
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
J. Leitao

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-1021MIS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00190	24/02/2000	24/02/1999
International Patent Classification (IPC) or national classification and IPC C12N15/49		
Applicant CONNAUGHT LABORATORIES LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/09/2000	08.06.2001
Name and mailing address of th international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Chavanne, F Telephon No. +49 89 2399 8399

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00190

I. Basis f th r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-18 as originally filed

Claims, No.:

1-16 with telefax of 13/03/2001

Drawings, sheets:

1/5-5/5 as originally filed

Sequence listing part of the description, pages:

1-7, filed with the letter of 02.05.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: -, which is: –

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (und r Rul 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, th international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
s separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether th claims are fully supported by the description, are made:
see separate sheet

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: AIDS research and human retroviruses
   Vol. 14, No. 2, pp. 151-155, 1998
   D2: Proc. Natl. Acad. Sci. USA
   Vol. 94, No. 17, pp. 9326-9331, 1997
   D3: WO 93/18055

2. D1 describes a vector comprising a sequence encoding gp140, the entire extracellular domain of HIV-1 Env, under the control of the cytomegalovirus promoter for expression of said gene construct in transfected cells (abstract; figure 1; page 152, column 2). An immunogenic composition comprising said vector was inoculated to rabbits by gene gun delivery after gold coating procedures to generate T-cell response to HIV-1 in said host (page 153, column 1, paragraph 2).

3. D2 discloses the *Env* gene and the Env protein of the primary HIV-1 isolate BX08 (abstract; page 9327, column 1, last paragraph; figure 1; page 9329, column 1). The extracellular fragment gp140 of Env proteins, and the nucleotide sequence coding for said fragment are known in the art. Since the sequence of the *Env* gene of the primary HIV-1 isolate BX08, and the amino acid sequence of the corresponding product are known in the art (see e.g. D2), the subject-matter of claim 16, which refers to the nucleotide sequence coding for the extracellular fragment gp140 of the Env protein of the primary HIV-1 isolate BX08 is not novel. Therefore, claim 16 does not meet the requirements of Article 33(2) PCT.

4. The subject-matter of claims 1-8, 10, 13 and 16 differs from the teaching of D1 in the sequence of the gp140. Said claims refer to the HIV-1 isolate BX08, whereas D1 refers to the HIV-1 isolate HXB-2.
   However, the primary isolate BX08 and its Env protein are known in the art and available (see e.g. D2). Since the cytotoxic response to HIV-1 of the immunogenic

composition of D1 depends on the gp140, it is obvious for the man skilled in the art, that the use of the gp140 from a different HIV-1 strain than the one used in D1 would allow the induction of a response directed to said different strain. Thus, the man skilled in the art, aware of D1 and D2, would not require any inventive skill to replace the sequence encoding the gp140 of D1 with the sequence of the gp140 form from the HIV-1 strain BX08. Thus, the subject-matter of claims 1-8, 10, 13 and 16 is not inventive.

The subject-matter of claim 9 further differs from D1 in the method used for delivery of the immunogenic composition. D1 mentions only gene gun delivery, whereas claim 9 refers to intramuscular immunization. However, the intramuscular delivery of immunogenic compositions is well-known in the art and routinely applied. By applying common knowledge, the man skilled in the art would automatically come to the subject-matter of claim 9. Thus, said subject-matter is not inventive.

The subject-matter of claims 11 and 12 further differs from D1 in that D1 does not specifically mention the generation of a cytotoxic T-cell response. However it is known in the art that peptides of the gp140 protein elicits a cytotoxic T lymphocyte response to HIV-1 Env (see e.g. D3, abstract; page 7, lines 11-15; page 11, lines 9-20). Thus, the man skilled in the art, also aware of D3, would not require any inventive skill to come to the subject-matter of claims 11 and 12. Thus, said subject-matter is not inventive.

Therefore, claims 1-13 and 16 do not meet the requirements of Article 33(3) PCT.

5. The subject-matter of claims 14 and 15 refers to peptides of gp140 of the primary HIV-1 isolate BX08. The sequence of said peptides have been determined using prediction algorithms for MHC class 1-restricted binding motifs (page 5, lines 3-7). However, the present application does not provide any experimental evidences showing that said peptides effectively elicit an immune response. The immunogenic activity of said peptides is pure speculation on the base of the result obtained with said algorithms. The present application show the induction of an immune response in the case of the gp140 protein only (see examples 4 and 5). Peptides characterised using prediction algorithms are not inventive. Thus, the subject-matter of claims 14 and 15 is not inventive.

Moreover, peptides of the gp140 protein eliciting an immune response are already known in the art (see D3, which discloses among others the peptide consisting of

the amino acid sequence of SEQ ID No. 3 of the present application). Since peptides of the gp140 protein of the HIV-1 isolate BX08 are already known in the art, unless the applicant can show unexpected properties for the claimed peptides, an inventive step cannot be acknowledged.
Therefore, claims 14 and 15 do not meet the requirements of Article 33(3) PCT.

VII. Certain defects in the international application

1. Claims 5-10 refer to an immunogenic composition. Claim 11 refers to a method of generating cytotoxic T-cell response to HIV-1 in a host. Claim 12 refers to the vector according to claim 1. Thus, it appears appropriate to move said claim 12 closer to claim 1.

VIII. Certain observations on the international application

1. Claims 3 and 4 lack clarity due to the expression "identifying characteristics" (Article 6 PCT). In fact, said expression is totally vague and unclear. It is totally subject to interpretation, and thus, is not adapted to define the scope of the claims. In the absence of any further indication, any plasmid may have the "identifying characteristics" of pCMV.gp140.BX08, pMP83, pMP84 or pMP88, depending on which parameter is taken into consideration.

2. Claims 4 and 8 lack clarity in that the expressions pMP83, pMP84 and pMP88 have no well-recognised meaning. They are internal designations, and thus, meaningless to a person skilled in the art (Article 6 PCT).

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

STEWART, Michael I.
Sim & McBurney
330 University Avenue
6th Floor
Suite 600
Toronto, Ontario M5G 1R7
CANADA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	08.06.2001
Applicant's or agent's file reference: 1038-1021MIS	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00190	24/02/2000	24/02/1999

Applicant
CONNAUGHT LABORATORIES LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith th international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of th report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of th PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	CLEERE, C T l.+49 89 2399-8061

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-1021MIS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00190	24/02/2000	24/02/1999
International Patent Classification (IPC) or national classification and IPC C12N15/49		
Applicant CONNAUGHT LABORATORIES LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/09/2000	08.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Chavanne, F Telephone No. +49 89 2399 8399

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00190

I. Basis of th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-18 as originally filed

Claims, No.:

1-16 with telefax of 13/03/2001

Drawings, sheets:

1/5-5/5 as originally filed

Sequence listing part of the description, pages:

1-7, filed with the letter of 02.05.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

V. Reason d stat m nt under Rul 66.2(a)(ii) with r gard to nov lty, inv ntive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: AIDS research and human retroviruses
   Vol. 14, No. 2, pp. 151-155, 1998
   D2: Proc. Natl. Acad. Sci. USA
   Vol. 94, No. 17, pp. 9326-9331, 1997
   D3: WO 93/18055

2. D1 describes a vector comprising a sequence encoding gp140, the entire extracellular domain of HIV-1 Env, under the control of the cytomegalovirus promoter for expression of said gene construct in transfected cells (abstract; figure 1; page 152, column 2). An immunogenic composition comprising said vector was inoculated to rabbits by gene gun delivery after gold coating procedures to generate T-cell response to HIV-1 in said host (page 153, column 1, paragraph 2).

3. D2 discloses the *Env* gene and the Env protein of the primary HIV-1 isolate BX08 (abstract; page 9327, column 1, last paragraph; figure 1; page 9329, column 1). The extracellular fragment gp140 of Env proteins, and the nucleotide sequence coding for said fragment are known in the art. Since the sequence of the *Env* gene of the primary HIV-1 isolate BX08, and the amino acid sequence of the corresponding product are known in the art (see e.g. D2), the subject-matter of claim 16, which refers to the nucleotide sequence coding for the extracellular fragment gp140 of the Env protein of the primary HIV-1 isolate BX08 is not novel. Therefore, claim 16 does not meet the requirements of Article 33(2) PCT.

4. The subject-matter of claims 1-8, 10, 13 and 16 differs from the teaching of D1 in the sequence of the gp140. Said claims refer to the HIV-1 isolate BX08, whereas D1 refers to the HIV-1 isolate HXB-2.
   However, the primary isolate BX08 and its Env protein are known in the art and available (see e.g. D2). Since the cytotoxic response to HIV-1 of the immunogenic

composition of D1 depends on the gp140, it is obvious for the man skilled in the art, that the use of the gp140 from a different HIV-1 strain than the one used in D1 would allow the induction of a response directed to said different strain. Thus, the man skilled in the art, aware of D1 and D2, would not require any inventive skill to replace the sequence encoding the gp140 of D1 with the sequence of the gp140 form from the HIV-1 strain BX08. Thus, the subject-matter of claims 1-8, 10, 13 and 16 is not inventive.

The subject-matter of claim 9 further differs from D1 in the method used for delivery of the immunogenic composition. D1 mentions only gene gun delivery, whereas claim 9 refers to intramuscular immunization. However, the intramuscular delivery of immunogenic compositions is well-known in the art and routinely applied. By applying common knowledge, the man skilled in the art would automatically come to the subject-matter of claim 9. Thus, said subject-matter is not inventive.

The subject-matter of claims 11 and 12 further differs from D1 in that D1 does not specifically mention the generation of a cytotoxic T-cell response. However it is known in the art that peptides of the gp140 protein elicits a cytotoxic T lymphocyte response to HIV-1 Env (see e.g. D3, abstract; page 7, lines 11-15; page 11, lines 9-20). Thus, the man skilled in the art, also aware of D3, would not require any inventive skill to come to the subject-matter of claims 11 and 12. Thus, said subject-matter is not inventive.

Therefore, claims 1-13 and 16 do not meet the requirements of Article 33(3) PCT.

5. The subject-matter of claims 14 and 15 refers to peptides of gp140 of the primary HIV-1 isolate BX08. The sequence of said peptides have been determined using prediction algorithms for MHC class 1-restricted binding motifs (page 5, lines 3-7). However, the present application does not provide any experimental evidences showing that said peptides effectively elicit an immune response. The immunogenic activity of said peptides is pure speculation on the base of the result obtained with said algorithms. The present application show the induction of an immune response in the case of the gp140 protein only (see examples 4 and 5). Peptides characterised using prediction algorithms are not inventive. Thus, the subject-matter of claims 14 and 15 is not inventive.

Moreover, peptides of the gp140 protein eliciting an immune response are already known in the art (see D3, which discloses among others the peptide consisting of

the amino acid sequence of SEQ ID No. 3 of the present application). Since peptides of the gp140 protein of the HIV-1 isolate BX08 are already known in the art, unless the applicant can show unexpected properties for the claimed peptides, an inventive step cannot be acknowledged.
Therefore, claims 14 and 15 do not meet the requirements of Article 33(3) PCT.

VII. Certain defects in the international application

1. Claims 5-10 refer to an immunogenic composition. Claim 11 refers to a method of generating cytotoxic T-cell response to HIV-1 in a host. Claim 12 refers to the vector according to claim 1. Thus, it appears appropriate to move said claim 12 closer to claim 1.

VIII. Certain observations on the international application

1. Claims 3 and 4 lack clarity due to the expression "identifying characteristics" (Article 6 PCT). In fact, said expression is totally vague and unclear. It is totally subject to interpretation, and thus, is not adapted to define the scope of the claims. In the absence of any further indication, any plasmid may have the "identifying characteristics" of pCMV.gp140.BX08, pMP83, pMP84 or pMP88, depending on which parameter is taken into consideration.

2. Claims 4 and 8 lack clarity in that the expressions pMP83, pMP84 and pMP88 have no well-recognised meaning. They are internal designations, and thus, meaningless to a person skilled in the art (Article 6 PCT).

CLAIMS

What we claim is:

1. A vector, comprising a gene encoding the extracellular fragment gp140 of the primary HIV-1 isolate BX08 under the control of a promotor for expression of the gene product in a host organism.

2. The vector of claim 1, wherein the promoter is the cytomegalovirus promotor.

3. The vector of claim 1, which has the identifying characteristics of pCMV.gp140.BX08 (ATCC No. 203839), as shown in Figure 1.

4. The vector of claim 1, which has the identifying characteristic of pMP83, pMP84 or pMP88

5. An immunogenic composition comprising a vector comprising a gene encoding the extracellular fragment gp140 of the primary HIV-1 isolate BX08 under the control of a promotor for expression of the gene product in a host organism.

6. The immunogenic compositions of claim 5, wherein the promoter is the cytomegalovirus promotor.

7. The immunogenic composition of claim 5, wherein the vector is pCMV.gp140.BX08.

8. The immunogenic composition of claim 5 wherein the vector is pMP83, pMP84 or pMP88.

9. The immunogenic composition of claim 5 formulated for intramuscular immunization with a pharmaceutically-acceptable liquid carrier.

10. The immunogenic composition of claim 6 formulated for gene gun delivery with gold particles.

11. A method of generating a cytotoxic T-cell response to HIV-1 in a host, which comprises administering to the host the immunogenic composition of claim 5

12. The vector according to claim 1 when used as an immunogen for generating a cytotoxic T-cell response to HIV-1 in a host.

13. The use of a vector according to claim 1 in the manufacture of an immunogen for generating a cytotoxic T-cell response to HIV1 in a host.

14. A peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID Nos.: 4 to 14, as shown in Table 1.

15. The peptide of claim 14, consisting of SEQ ID No.:5.

16. A nucleic acid molecule consisting of a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence consisting of SEQ ID No: 1 or the complementary sequence thereto;

(b) a nucleotide sequence encoding a gp140 protein consisting of SEQ ID No: 2 or the complementary sequence thereto; and

(c) a nucleotide sequence consisting of SEQ ID No: 15 or the complementary sequence thereto.

TENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
1038-1021MIS		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00190	24/02/2000	24/02/1999
Applicant		
CONNAUGHT LABORATORIES LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/CA 00/ 00190

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

[redacted]tional Application No
[redacted]/CA 00/00190

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/49 C07K14/16 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LU S ET AL: "Immunogenicity of DNA vaccines expressing human immunodeficiency virus type 1 envelope glycoprotein with and without deletions in the V1/2 and V3 regions." AIDS RESEARCH AND HUMAN RETROVIRUSES, JAN 20 1998, 14 (2) P151-5, XP000907375 UNITED STATES	1,3,6,8, 11,15
Y	abstract; figure 1	2,4,7,9
X	WO 93 18055 A (US HEALTH) 16 September 1993 (1993-09-16) page 7, line 16; claims 1,5,12,28	16,17
X	WO 97 31115 A (DAVIES MARY ELLEN ;PERRY HELEN C (US); SHIVER JOHN W (US); FREED D) 28 August 1997 (1997-08-28)	1,3,15
A	claims 1-10; figure 1; example 7	6,8,11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2000	14/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	YAHI N ET AL: "STRUCTURAL VARIABILITY OF ENV AND GAG GENE PRODUCTS FROM A HIGHLY CYTOPATHIC STRAIN OF HIV-1" ARCHIVES OF VIROLOGY 1992, vol. 125, no. 1-4, 1992, pages 287-298, XP000907410 ISSN: 0304-8608	1,3,15
A	the whole document	6,8,11
Y	VERRIER FLORENCE C ET AL: "Antibodies to several conformation-dependent epitopes of gp120/gp41 inhibit CCR-5-dependent cell-to-cell fusion mediated by the native envelope glycoprotein of a primary macrophage-tropic HIV-1 isolate." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1997, vol. 94, no. 17, 1997, pages 9326-9331, XP002138893 ISSN: 0027-8424 abstract; figure 1	2,4,7,9
A	WO 94 28929 A (GENENTECH INC ;BERMAN PHILLIP W (US); NAKAMURA GERALD R (US)) 22 December 1994 (1994-12-22) page 32 -page 35; claims 1-33	1-7,15, 19
A	O'BRIEN W A ET AL: "HIV-1 TROPISM FOR MONONUCLEAR PHAGOCYTES CAN BE DETERMINED BY REGIONS OF GP120 OUTSIDE THE CD4-BINDING DOMAIN" NATURE (LONDON) 1990, vol. 348, no. 6296, 1990, pages 69-73, XP002138883 ISSN: 0028-0836 the whole document	1-7,15, 19
A	-& EMBL DATABASE ; ACCESSION NUMBER U63632,30 August 1996 (1996-08-30), XP002138884 figure GP160	1-7,15, 19

-/--

4

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WESTERVELT P ET AL: "IDENTIFICATION OF A DETERMINANT WITHIN THE HUMAN IMMUNODEFICIENCY VIRUS 1 SURFACE ENVELOPE GLYCOPROTEIN CRITICAL FOR PRODUCTIVE INFECTION OF PRIMARY MONOCYTES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1991, vol. 88, no. 8, 1991, pages 3097-3101, XP002138885 ISSN: 0027-8424 the whole document	1-7,15, 19
A	-& EMBL DATABASE ; ACCESION NUMBER M60472, 1 May 1991 (1991-05-01), XP002138886 the whole document	1-7,15, 19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/CA 00/00190

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9318055 A	16-09-1993	US 5976541 A	02-11-1999
		AU 668927 B	23-05-1996
		AU 3787893 A	05-10-1993
		CA 2131153 A	16-09-1993
		EP 0630385 A	28-12-1994
		US 5932218 A	03-08-1999
WO 9731115 A	28-08-1997	AU 2124697 A	10-09-1997
		BG 102784 A	31-05-1999
		BR 9707672 A	13-04-1999
		CN 1216064 A	05-05-1999
		CZ 9802667 A	17-03-1999
		EP 0904380 A	31-03-1999
		HR 970092 A	30-04-1998
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		NO 983876 A	21-10-1998
		PL 328730 A	15-02-1999
WO 9428929 A	22-12-1994	AU 700371 B	07-01-1999
		AU 7047894 A	03-01-1995
		EP 0708659 A	01-05-1996
		NZ 267838 A	19-12-1997
		US 6042836 A	28-03-2000
		US 5864027 A	26-01-1999

CLAIMS

What we claim is:

1. A vector, comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promotor for expression of the gene product in a host organism.

2. The vector of claim 1, wherein said primary HIV-1 isolate is BX08.

3. The vector of claim 2, wherein the promoter is the cytomegalovirus promotor.

4. The vector of claim 1, which has the identifying characteristics of pCMV.gp140.BX08 (ATCC No. 203839), as shown in Figure 1.

5. The vector of claim 1, which has the identifying characteristic of pMP83, pMP84 or pMP88.

6. An immunogenic composition comprising a vector comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promotor for expression of the gene product in a host organism.

7. The immunogenic compositions of claim 6, wherein said primary HIV-1 isolate is BX08.

8. The immunogenic compositions of claim 6, wherein the promoter is the cytomegalovirus promotor.

9. The immunogenic composition of claim 6, wherein the vector is pCMV.gp140.BX08.

10. The immunogenic composition of claim 6 wherein the vector is pMP83, pMP84 or pMP88.

11. The immunogenic composition of claim 6 formulated for intramuscular immunization with a pharmaceutically-acceptable liquid carrier.

12. The immunogenic composition of claim 6 formulated for gene gun delivery with gold particles

13. A method of generating a cytotoxic T-cell response to HIV-1 in a host, which comprises administering to the host the immunogenic composition of claim 6.

14. The vector according to claim 1 when used as an immunogen for generating a cytotoxic T-cell response to HIV-1 in a host.

15. The use of a vector according to claim 1 in the manufacture of an immunogen for generating a cytotoxic T-cell response to HIV1 in a host.

16. A peptide having an amino acid sequence selected from the group consisting of SEQ ID Nos.: 3 to 14, as shown in Table 1.

17. The peptide of claim 16, having SEQ ID No.:3.

18. The peptide of claim 16, having SEQ ID No.:5.

19. A nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence having SEQ ID No: 1 or the complementary sequence thereto;

(b) a nucleotide sequence encoding a gp140 protein having SEQ ID No: 2 or the complementary sequence thereto; and

(c) a nucleotide sequence having SEQ ID No: 15 or the complementary sequence thereto.

EXHIBIT A

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7: C12N 15/49, C07K 14/16, A61K 48/00 — A1

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(30) Priority Data:
09/256,194 24 February 1999 (24.02.99) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/256,194 (CIP)
Filed on 24 February 1999 (24.02.99)

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(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SIA, Charles, D., Y. [CA/CA]; 189 Mabley Crescent, Thornhill, Ontario L4J 2Z7 (CA). CAO, Shi-Xian [CA/CA]; Apt. 408, 716 The West Mall, Etobicoke, Ontario M9C 4X6 (CA). PERSSON, Roy [CA/CA]; Unit 604, 7 Bishop Avenue, North York, Ontario M2M 4J4 (CA). ROVINSKI, Benjamin [CA/CA]; 70 Winding Lane, Thornhill, Ontario L4J 5H6 (CA). PARRINGTON, Mark [CA/CA]; 45 Martin Street, Bradford, Ontario L3Z 1Z4 (CA).

(74) Agent: STEWART, Michael, I.; Sim & Mcburney, 6th Floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).

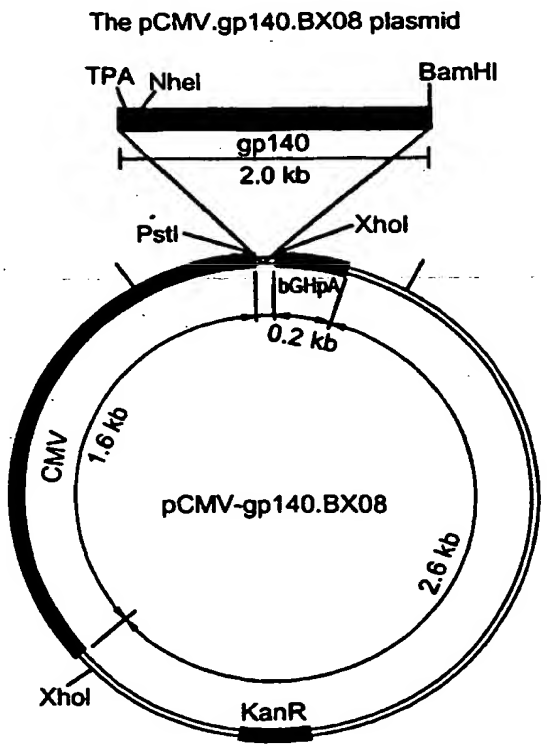
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: EXPRESSING GP140 FRAGMENT OF PRIMARY HIV-1 ISOLATE

(57) Abstract

A vector for eliciting an immune response to a host comprising a gene encoding the gp140 protein of the primary isolate of HIV-1, BX08, under the control of a promotor for expression of the protein in the host, specifically plasmids pCMV.gp140.BX08, pMP83, pMP84 and pMP88. Murine and human MHC class 1-restricted binding motifs contained in BX08 are identified.



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TITLE OF INVENTION

EXPRESSING GP140 FRAGMENT OF PRIMARY HIV-1 ISOLATE

FIELD OF INVENTION

The present invention relates to the field of immunology, specifically HIV Vaccine Technology, and, in particular, is concerned with expressing the extracellular fragment of the envelope gene, gp140, of a primary human immunodeficiency virus type 1 (HIV-1) isolate.

REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending United States Patent Application No.: 09/256,194 filed February 24, 1999.

BACKGROUND OF THE INVENTION

Acquired immunodeficiency syndrome (AIDS) is a disease which is the ultimate result of infection with human immunodeficiency virus (HIV). Currently, there is no effective vaccine which can protect the human population from HIV infection and hence the development of an efficacious HIV-vaccine and protocol for administering the same is urgently required. Previously, HIV-1 particles exhaustively inactivated by chemical treatments, a vaccinia vector encoding the whole envelope gene (gp140) of HIV-1, and purified recombinant gp120 have been evaluated as candidate HIV vaccines. Although inactivated HIV-1 virus preparations elicited a T-cell-mediated Delayed-Type Hypersensitivity (DTH) reaction in humans, and vaccinia/gp160 and gp120 recombinant vaccine candidates induced virus neutralizing antibodies, none of these immunogens have been shown to be efficacious human HIV vaccines (ref. 1, throughout this specification, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately following the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). The inventors' interest in HIV vaccinology is to develop immunogenic and cost-effective HIV-1 DNA vaccines and consider that

their use alone or in conjunction with other forms of HIV-1 vaccine candidates will lead to the elicitation of more effective immune responses against HIV-1.

There has previously been described in granted European Patent No. 470,980 and U.S. Patent No. 5,639,854, assigned to the assignee hereof, the disclosures of which are incorporated herein by reference, *inter alia*, the identification and characterization of a T-cell epitope of the core protein, p24E, of HIV-1. There has further been described in granted U.S. Patents Nos. 5,759,769 and 5,795,955, assigned to the assignee hereof, and disclosures of which are incorporated by reference, the use of the T-cell epitope in the construction of immunogenic synthetic HIV-1 chimeric peptides comprising p24E linked to amino acid sequences of different B-cell epitopes of an envelope or core protein of HIV-1.

SUMMARY OF THE INVENTION

The present effort has turned to design and construction of HIV DNA-based immunogens capable of eliciting cell-mediated immunity (CMI). In this context, the inventors have focused interest on the extracellular envelope fragment, gp140, expressed in a primary HIV-1 isolate, HIV-1 (BX08), for the reason that this protein is rich in motifs restricted to both the murine and human Major Histocompatibility Complex (MHC) class 1 alleles.

Immunization with an appropriately constructed immunogen expressing the gp140 protein leads to the generation of peptides with class 1 binding capability to allow the induction of HIV-1-specific CTLs capable of killing virus infected cells to limit infection.

The invention described by the inventors is that they have found a plasmid designated, pCMV.gp140.BX08, expressing the gp140 gene under the control of a CMV promotor was immunogenic in BALB/c mice in the elicitation of CTL response directed against multiple epitopes of the gp140 protein that are restricted to different H-2^d class 1 gene products. It was also found that plasmids based on Semliki Forest Virus (SFV) vectors, namely pMP83, pMP84 and pMP88, also requiring the gp140 gene under the control of a CMV promoter were similarly immunogenic.

Accordingly, in one aspect of the present invention, there is provided a vector, comprising a gene encoding the extracellular fragments of gp140 of a primary HIV-1 isolate, preferably BX08, under the control of a promotor for expression of the gene product in a host organism, thereby eliciting a cytotoxic T-cell response.

The promotor preferably is the cytomegalovirus promotor. The vector may preferably be a plasmid vector having the identifying characteristics of plasmid pCMV.gp140.BX08, as shown in Figure 1. The vector also may preferably be a plasmid vector having the identifying characteristics of plasmids pMP88, pMP84 or pMP83.

The invention further includes an immunogenic composition containing the vector as well as a method of generating a cytotoxic T-cell response to HIV-1 in a host by administering to the host the immunogenic composition provided herein. Such immunogenic composition may be formulated for intramuscular immunization with a suitable carrier or may be formulated for gene gun delivery with gold particles.

The invention extends to the vector when used as an immunogen for generating a cytotoxic T-cell response to HIV-1 in a host and to the use of the vector in the manufacture of an immunogen for the generation of a cytotoxic T-cell response to the HIV-1 in a host.

The invention further includes certain novel peptides and nucleic acid molecules as set forth below.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows details of the elements of plasmid pCMV.gp140.BX08;

Figure 2 shows the nucleotide (SEQ ID No: 1) and deduced amino acid sequence (SEQ ID No: 2) of the gp140 open reading frame of the plasmid pCMV.gp140.BX08;

Figures 3A, 3B and 3C show the effector responses elicited by intramuscular injection of the plasmid, pCMV.gp140.BX08, in BALB/c mice;

Figures 4A, 4B and 4C show the effector responses elicited by gene gun delivery of the plasmid, pCMV.gp140.BX08, in BALB/c mice; and

Figure 5 is a nucleotide sequence of a BamHI fragment (SEQ ID No: 15) containing the HIV-1, gp140 nucleotide sequence for incorporation into a BamHI site of alphavirus expression vectors.

DETAILED DESCRIPTION OF THE INVENTION

A DNA immunogen is constructed using recombinant DNA technology to molecularly clone a gene of interest into a plasmid expression vector. A unique feature involving vaccination with a DNA-based immunogen is that, once delivered into a cell, the intracellular production of the immunogen favours the induction of MHC class 1-restricted cytotoxic T-cells as compared to other forms of vaccination involving the use of killed whole cell and formulated sub-unit immunogens, which tend to favour the elicitation of MHC class 2-restricted immuno-regulatory responses in the majority of cases studied (ref. 2). In this context, it is, therefore, favourable to use DNA technology to construct naked DNA immunogens for vaccination purposes in order to optimize the induction of cellular effector response against intracellular organisms, such as viruses as well as certain tumours. The other advantages DNA vaccines offer include: (i) the ease to produce them; and (ii) their stability over a wide temperature range.

A common model which has been used recently to predict murine and human CTL antigenic determinants has involved the identification of binding motifs for the respective MHC class 1 molecules from the primary sequences of the native protein molecules (see refs. 3 to 5). Thus, it has been proposed that motifs which are most favoured to bind and lodge into the peptide-binding groove of the H-2D^d gene product is usually 8 to 10 amino acids long. In the majority of cases, these peptides are found to contain anchor residues, such as glycine and proline (GP), at positions 2 and 3 near the amino- (N-) terminus, and either a leucine or phenylalanine at the carboxy- (C-) terminus, which serve to interact with the respective 'pockets' of the peptide-binding groove of a membrane-bound H-2D^d molecule. The motifs restricted to the other class 1 allele, K^d, of the H-2^d haplotype were reported to contain a tyrosine at position 2, and could be an isoleucine, valine or leucine at the C-terminus. Studies of the peptides isolated from the human MHC class 1 molecules, HLA-A0201, had similarly revealed that

the anchor residues were leucine or methionine at position 2 and valine or leucine at the C-terminus in the majority of cases.

The suitability of the HIV-1(BX08) gp140 gene product as a CTL-inducing immunogen was assessed by prediction algorithms to determine the number of both the murine and human MHC class 1-restricted binding motifs it contained. The amino acid sequences of the binding motifs and the designation of the peptides representing them are shown in Table 1 below. Such peptides are novel and are claimed herein. The presence of binding motifs towards the different H-2^d restricted class 1 alleles, i.e. D^d and K^d, allows the immunogenicity of plasmids, pCMV.gp140.BX08, pMP83, pMP84 and pMP88, expressing gp140 of HIV-1 of the primary isolate, BX08, and constructed as described in the Examples below, to be studied in the inbred mouse strain BALB/c of the H-2^d haplotype. The elements and restriction sites of plasmid pCMV.gp140.BX08 are shown in Figure 1. The construction of the plasmids pMP83, pMP84 and pMP88 is described in Example 5 below. The nucleotide sequence (SEQ ID No: 1) and the deduced amino acid sequence (SEQ ID No: 2) of the gp140 open reading frame of the plasmid pCMV.gp140.BX08, pMP83, pMP84 and pMP88, is shown in Figure 2, which appear to be unique sequences and are claimed herein along with their complements.

The location of several binding motifs against the human MHC class 1 allele, HLA-A0201, as seen in Table 1, implied that, under an appropriate immunization regimen, the plasmid has the potential to elicit CTL response directed to these epitopes in the context of this class 1 molecule in human subjects.

The immunogenicity of the plasmid pCMV.gp140.BX08 was studied in BALB/c mice. The results of the study involving three injections of the plasmid at 100.0 μg per dose using the intramuscular route are shown in Figure 3. Upon *in vitro* re-stimulation of the spleenocytes of the plasmid-immunized animals with irradiated autologous LPS blasts pulsed individually with the D^d- and K^d-restricted motif containing peptides, namely, CLP-501 and CLP-504 (SEQ ID Nos.: 3, 5), respectively, it was found that CTLs were generated that killed P815 targets presented with the respective peptides (Figs. 3A and 3B). The amino acid

sequences of the peptides are shown in Table 1. A comparison of the magnitude of the responses at the same effector to target (E:T) ratio revealed that the D^d-restricted response to the CLP-501 peptide is immuno-dominant and that the K^d-restricted response to the CLP-504 peptide is sub-dominant. The *in vitro* re-stimulation leading to the expansion of the effectors was specific because the addition of the same number of irradiated LPS blasts alone (not treated with peptide) did not lead to any generation of effectors in the bulk culture able to kill either of the specific targets tested. The findings that the control group of mice injected with the pCMV vector without the gp140 insert alone failed to generate any of the two sub-populations of CTLs (Fig. 3C) confirmed that the plasmid, pCMV.gp140.BX08, was indeed immunogenic.

The pCMV.gp140.BX08 plasmid, when delivered with the gene gun, was similarly found to be immunogenic. The results shown in Figure 4 show that following two injections at a dose of 0.7 μg of the plasmid, and using the same *in vitro* re-stimulation condition described above that CTLs recognizing the CLP-501 and CLP-504 peptides were detected (Figures 4A and 4B), while no effector response was elicited by the group of animals given the vector, pCMV, alone (Figure 4C).

The immunogenicity of the plasmids pMP83, pMP84 and pMP88 was separately studied, delivered intramuscularly, in BALB/c mice, in comparison to pCMV.gp140.BX08, at three different dosage levels, namely 1.0, 10.0 and 100.0 μg of DNA following the above described procedures. The results obtained are contained in Tables II and III below. CTL activation is achieved at significantly lower doses of the alphavirus vectors than with pCMV.gp140.BX08.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of HIV infections. A further non-limiting discussion of such uses is further presented below.

Immunogenic compositions, including vaccines, containing the DNA vector may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The DNA vector may be associated with liposomes, such as lecithin liposomes or other liposomes

known in the art, as a nucleic acid liposome (for example, as described in WO 93/24640) or the DNA vector may be associated with an adjuvant, as described in more detail below.

Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides.

Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used with the vector.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particular carrier of a phospholipid/glycolipid/ polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain and absorption enhancer.

In particular embodiments of the present invention, the vector may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The vectors may be delivered to the host by a variety of procedures, for example, Tang et al (ref. 6) discloses that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 7) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

Biological Deposits

Certain vectors that contain nucleic acid coding for an extracellular fragment of gp140 of a primary isolate that are described and referred to herein as well as precursor alphavirus vectors have been deposited with the America Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA, pursuant the Budapest Treaty and prior to the filing of this application. Samples of the deposited vectors will become available to the public and all restrictions imposed or access to the deposits will be received upon grant of a patent based on this United States patent application. In addition, the deposit will be replaced if viable samples cannot be dispensed by the Depository. The invention described and claimed herein is not limited in scope by the biological materials deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar vectors that contain nucleic acid which encodes equivalent or similar antigens as described in this application are within the scope of the invention.

Deposit Summary

Plasmid	ATCC	Deposited Date
pCMV.gp140.BX08	203839	March 9, 1999
pMP42	203461	November 12, 1998
pMP76	203462	November 12, 1998

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific

Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Methods of plasmid construction, peptide synthesis, cell culture, CTL assay and other testing procedures that are not explicitly described in this disclosure are amply reported in the scientific literature and are well within the scope of those skilled in the art.

Example 1

This Example illustrates the construction of the plasmid, pCMV.gp140.BX08.

The plasmid, pCMV.gp140.BX08, contains sequence segments from various sources, and the elements of construction are depicted in Figure 1. The nucleotide (SEQ ID No: 1) and derived amino acid sequences (SEQ ID No: 2) of the gp140 open reading frame of the plasmid are shown in Figure 2.

The prokaryotic vector pBluescript SK (Stratagene) is the backbone of the plasmid pCMV.gp140.BX08 and was modified by the replacement of the Amp^R with Kan^R gene and the deletion of the f1 and the LacZ regions. To achieve the desired modifications, the sequence between AhdI (nucleotide 2,041) and SacI (nucleotide 759) of pBluescript SK, which contains the Amp^R, f1 origin and the LacZ, was deleted. A 1.2 kb PstI fragment from the plasmid pUC-4K (Pharmacia) containing the Kan^R gene, was blunt end ligated to the AhdI site of pBluescript SK in a counter-clockwise orientation relative to it's transcription. A 1.6 kb SspI/PstI DNA fragment containing the human cytomegalovirus immediate-early gene promotor, enhancer and intron A sequences (CMV) was ligated to the other end of the Kan^R gene so that the transcription from the CMV promotor proceeds in the clockwise orientation. A synthetic oligonucleotide segment containing translation initiation sequence and sequences encoding the human tissue plasminogen activator signal peptide (TPA) was used to link the CMV promotor and the sequences encoding the gp140 of the primary isolate $HIV\text{-}1_{BX08}$. The gp140 sequence encodes a portion of the envelope protein between amino acid 33 and

666 which ends before the transmembrane domain of gp41 (see Figure 2). A translation termination codon was placed at the end of the gp140 coding sequence. Next to the gp140 coding region is a 0.2 kb fragment containing the bovine growth hormone (BGH) polyadenylation signal sequence that is PCR amplified from pRC/CMV (Invitrogen). A remnant 80 bp DNA segment from the SV40 polyadenylation signal remained between the BGH poly A sequence and the SacI site of pBluescript SK due to DNA manipulation and it serves no purpose in this plasmid.

The pCMV.gp140.BX08 construct was introduced into HB101 competent cells according to manufacturer's recommendations (GibcoBRL). Correct molecular clones were identified by restriction and sequencing analysis and their expression of gp140 was examined in transient transfections followed by Western blot analysis.

All DNAs used for immunizations were prepared using EndoFree Plasmid Kit (Qiagen). For intramuscular immunizations in mice, 100 μg of pCMV.gp140.BX08, in 100 μl PBS was injected into the tibialis anterior muscles at 4 weeks intervals. Gene gun immunizations were accomplished with the Helios Gene Gun System (Biorad). Cartridges were prepared according to manufacturer's recommendations. Specifically, each cartridge was made to contain 0.7 μg of the DNA and 0.5 mg gold. Immunizations were carried out by applying two cartridges to each animal onto the shaved abdominal area at 4 week intervals.

Example 2

This Example illustrates the synthesis of peptides.

Solid phase peptide synthesis of peptide CLP-501 and CLP-504 were conducted on an ABI 430A automated peptide synthesizer according to the manufacturer's standard protocols. The peptides were cleaved from solid support by treatment with liquid hydrogen fluoride in the presence of thiocresole, anisole, and methyl sulfide. The crude products were extracted with trifluoroacetic acid (TFA) and precipitated with diethyl ether.

The amino acid sequences of these peptides are shown in Table 1.

Example 3

This Example illustrates *in vitro* cell culture protocols to re-stimulate and expand CTLs and assay for their effector functions.

Spleens of BALB/c mice injected with the plasmid, pCMV.gp140.BX08, prepared and formulated as described in Example 1, using the intramuscular route or gene gun delivery method, were removed 10 to 11 days post final booster injection. Spleenocytes at 3.0×10^7 were co-cultured with 1.3×10^7 autogolous LPS blasts which had been pulsed with the test peptide for 5hr at 37°C and irradiated at 3000 rads in 10.0 ml of complete medium (RPMI 1640 supplemented with 10.0% 56°C heat-inactivated bovine serum, 120.0 units per ml of penicillin G sodium, 120.0 µg per ml of streptomycin sulphate and 0.35 mg per ml of L-glutamine) in a 25 cm^2 tissue culture flask. The cultures were kept at 37°C in a humidified CO_2 incubator for days, and the responders were then tested against peptide-pulsed P815 target cells in a standard *in vitro* 4 hr CTL assay as follows:

The responders were harvested from the 7-day cultures and washed once with RPMI 1640 medium without added bovine serum. The positive target was created by incubating 3 to 5×10^6 P815 cells with 100.0 µg of the specified peptide overnight in a 26°C water bath. The target cells were then labeled with ^{51}Cr at 250.0 uCi per 1×10^6 cells in the presence of 25.0 µg of the same test peptides for 60 to 75 minutes at 26°C. After washing twice with complete medium to remove excess ^{51}Cr, the targets were incubated at 2.5×10^3 with different numbers of the responders per well in a V-bottomed 96 will tissue culture plates for 4 hr in a 37°C $C0_2$ incubator. Half amount of the supernatant from each micro-assay culture was then removed and counted for radioactivity. Results were expressed as % which was calculated using the equation:

% lysis = (spontaneous lysis in cpm of experimental sample – spontaneous lysis in cpm of labeled target cells alone) divided by (total lysis in cpm of target cells alone – spontaneous lysis in cpm of target cells alone) x 100.

The results obtained employing intramuscular injection are shown in Figures 3A, 3B and 3C while those obtained employing the gene gun delivery are shown in Figures 4A, 4B and 4C.

Example 4

This Example illustrates the construction of alphavirus expression vectors pMP83, pMP84 and pMP88.

DNA vector pCMV.gp140.BX08, prepared as described in Example 1, was digested with restriction endonucleases NbeI and BamHI to release the HIV-1 gp140 sequence. The NheI/BamHI fragment was gel purified and ligated to a synthetic oligonucleotide linker made from the following annealed oligonucleotides: Oligo 1 - TPA-1

5'- TCC GGA TCC ACC ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG G -3' (SEQ ID No: 16)

Oligo 2 - TPA-2

5'- CTA GTC GAA ACG AAG ACT GCT CCA CAC AGC AGC AGC ACA CAG CAG AGC CCT CTC TTC ATT GCA TCC ATG GTG GAT CCG GA -3' (SEQ ID No: 17). This ligation restrored the TPA signal sequence. The resulting fragment was restricted with BamHI and the resulting BamHI fragment was gel purified. The nucleotide sequence of the BamHI fragment is shown in Figure 5 (SEQ ID No: 15).

After the DNA sequence verification, the BamHI fragment was cut out of the pUC19 vector and ligated into BamHi restricted plasmids pMP42, pMP74 and pMP76 to make plasmids pMP88, pMP84 and pMP83 respectively.

Plasmid pMP42, containing the SFV replicon, is described in WO 99/25858 (1038-864), assigned to the applicants and the disclosure of which is incorporated herein by reference and has been deposited with ATCC (203461). The construction of pMP42 is shown in Figures 2A and 2B of WO 99/25858.

Plasmid pMP76, containing the SFV replicon, is described in WO 99/25859 (1038-862), assigned to the applicants and the disclosure of which is incorporated herein by reference and has been deposited with ATCC (203462). The construction of pMP76 is shown in Figures 8A to 8D of WO 99/25859.

Plasmid pMP74, containing the SFV replicon, is identical to pMP76 except that it lacks the rabbit β-globin intron II insertion into the SFV replicon. This plasmid may be constructed by suitable modification to the scheme shown in Figures 8A to 8D of WO 99/25859.

Example 5

This Example shows the results of immunizations using the alphavirus vectors.

The recombinant alphavirus vectors pMP88, pMP84 and pMP83, prepared as described in Example 4, were employed in comparative immunogenicity studies with plasmid pCMV.gp140.BX08, prepared as described in Example 1, in BALB/c mice following the procedure outlines in Example 1 for intramuscular immunization in mice using pCMV.gp140.BX08 and the CTL assay of Example 3, with unmodified pMP76 and pCMV being employed as negative controls. The results obtained are shown in Table II below.

Comparative analysis of the alphavirus constructs and the DNA construct of Example 1 showed similar results in the CTL assay. As expected, the negative control vectors that did not contain the gp140 sequences from HIV-1 BX08 showed no specific lysis in the CTL assay. All three alphavirus replicons, pMP83, pMP84 and pMP88, showed specific lysis as did the vector pCMV.gp140.BX08. The difference between the two types of vector was the amount of immunizing nucleic acid needed to elicit the same response. At 1 μg dose, the alphavirus vectors pMP83 and pMP88 showed comparable responses to pCMV.gp140.BX08 at a much higher dose of 100 μg.

These results were confirmed by an vector from a interferon-gamma (IFN-γ) assay, the results of which are shown in Table III. The assay is well known, as the measure of IFN-γ secreted from the spleenocytes indicated activation of the CTLs. Again, the alphavirus vectors showed comparable activation at an approximately 100 fold lower dose than the pCMV.gp140.BX08 vector. Overall these results indicate that immunization with nucleic acid vector expressing the gp140 sequence from the primary isolate BX08 generated MHC Class I restricted cytotoxic T-cells and that the alphavirus expression system used was approximately 100-fold more effective at the lower dose.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a novel plasmid expressing, *in vitro* and *in vivo*, the gp140 protein of the primary HIV-1

isolate BX08 and the generation of MHC class 1-restricted cytotoxic T-cells in animals. Modifications are possible within the scope of this invention.

Table 1

MHC class 1-restricted motifs of the extracellular envelope fragment, gp140, of HIV-1(BX08)

H-2^d-restricted *		HLA-A0201-restricted **		
Peptide ***	D^d-	Peptide ***	K^d-	
CLP-501	**IGPGRAFYTT** (274-283) (SEQ ID No:3)	CLP-503	AYDTEVHNV (29-37) (SEQ ID No:4)	KLTPLCVTL (91-98) (SEQ ID No:10)
		CLP-504	FYSLKIVPI (141-149) (SEQ ID No:5)	TLFRVAIKL (305-313) (SEQ ID No:11)
		CLP-505	LYKYKVVKI (443-451) (SEQ ID No:6)	TLTVQARQL (403-411) (SEQ ID No:12)
		CLP-506	KYKVVKIEPL (445-454) (SEQ ID No:7)	TLTVQARAL (496-504) (SEQ ID No:13)
		CLP-507	RYLQDQRFL (545-553) (SEQ ID No:8)	QLQARVLAL (535-543) (SEQ ID No:14)
		CLP-508	NYTEIIYSL (597-605) (SEQ ID No:9)	

* or ** Anchors residues were typed in bolded letters.

*** Peptides chosen for the study reported herein are bolded.

Table II

A comparative immunogenicity study of recombinant alpha viruses and pCMV.gp140.BX08 expressing gp140 of HIV-1_{BX08} in BALB/c mice

Immunization (3 injections, im)	Dose (μg)	% CLP-501 specific lysis at E:T ratio of 75:1
pMP76	100.0 10.0 1.0	0.6 0.02 0
pMP83	100.0 10.0 1.0	23.4 38.5 42.7
pMP84	100.0 10.0 1.0	37.2 18.6 12.4
pMP88	100.0 (not done, mice died) 10.0 1.0	 39.6 49.6
pCMV3	100.0 10.0 1.0	0 0.06 0
PCMV.gp140.BX08	100.0 10.0 1.0	59.7 29.3 0

Table III

Frequencies of spleenic CLP-501-specific effectors induced by recombinant vectors expressing HIV-1_{BX08} gp140 determined by IFN-gamma ELISPOT

Immunization (3 injections, im)	Dose (μg)	Number of spots scored per $5X10^5$ responders In duplicate cultures
pMP76	100.0 10.0 1.0	0, 0 0, 0 0, 0
pMP83	100.0 10.0 1.0	10, 14 14, 19 18, 13
pMP84	100.0 10.0 1.0	7, 12 4, 7 2, 6
pMP88	100.0 (not done, mice died) 10.0 1.0	20, 13 24, 17
pCMV3	100.0 10.0 1.0	0 0 0
PCMV.gp140.BX08	100.0 10.0 1.0	17, 13 6, 9 0

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CLAIMS

What we claim is:

1. A vector, comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promotor for expression of the gene product in a host organism.

2. The vector of claim 1, wherein said primary HIV-1 isolate is BX08.

3. The vector of claim 2, wherein the promoter is the cytomegalovirus promotor.

4. The vector of claim 1, which has the identifying characteristics of pCMV.gp140.BX08 (ATCC No. 203839), as shown in Figure 1.

5. The vector of claim 1, which has the identifying characteristic of pMP83, pMP84 or pMP88.

6. An immunogenic composition comprising a vector comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promotor for expression of the gene product in a host organism.

7. The immunogenic compositions of claim 6, wherein said primary HIV-1 isolate is BX08.

8. The immunogenic compositions of claim 6, wherein the promoter is the cytomegalovirus promotor.

9. The immunogenic composition of claim 6, wherein the vector is pCMV.gp140.BX08.

10. The immunogenic composition of claim 6 wherein the vector is pMP83, pMP84 or pMP88.

11. The immunogenic composition of claim 6 formulated for intramuscular immunization with a pharmaceutically-acceptable liquid carrier.

12. The immunogenic composition of claim 6 formulated for gene gun delivery with gold particles

13. A method of generating a cytotoxic T-cell response to HIV-1 in a host, which comprises administering to the host the immunogenic composition of claim 6.

14. The vector according to claim 1 when used as an immunogen for generating a cytotoxic T-cell response to HIV-1 in a host.

15. The use of a vector according to claim 1 in the manufacture of an immunogen for generating a cytotoxic T-cell response to HIV1 in a host.

16. A peptide having an amino acid sequence selected from the group consisting of SEQ ID Nos.: 3 to 14, as shown in Table 1.

17. The peptide of claim 16, having SEQ ID No.:3.

18. The peptide of claim 16, having SEQ ID No.:5.

19. A nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence having SEQ ID No: 1 or the complementary sequence thereto;

(b) a nucleotide sequence encoding a gp140 protein having SEQ ID No: 2 or the complementary sequence thereto; and

(c) a nucleotide sequence having SEQ ID No: 15 or the complementary sequence thereto.

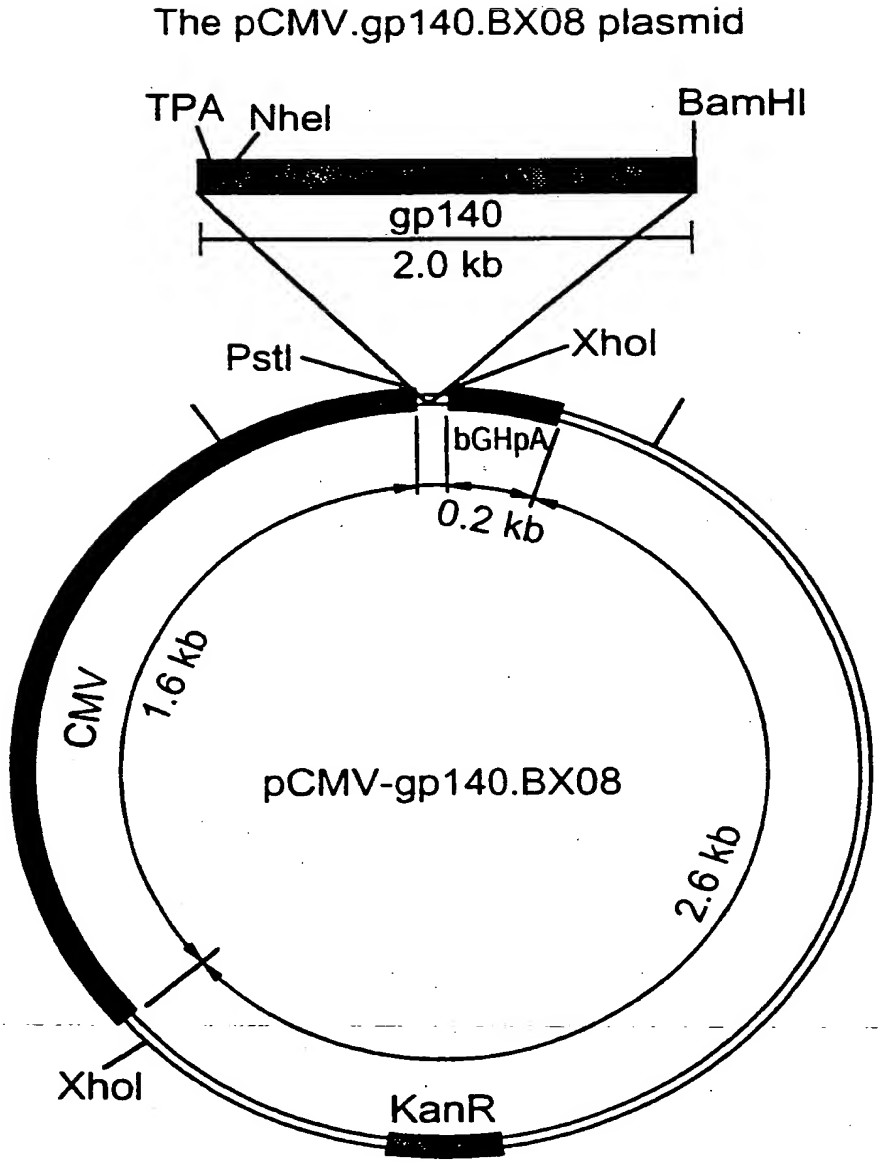
The pCMV.gp140.BX08 plasmid
TPA
NheI
BamHI
gp140
2.0 kb
PstI
XhoI
bGHpA
0.2 kb
CMV
1.6 kb
pCMV-gp140.BX08
2.6 kb
XhoI
KanR

FIG.1

FIG.2A

```
+1  M  D  A  M  K  R  G  L  C  C  V  L  L  L  C  G
1   ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA
    TAC CTA CGT TAC TTC TCT CCC GAG ACG ACA CAC GAC GAC GAC ACA CCT

+1  A  V  F  V  S  A  S  L  W  V  T  V  Y  Y  G  V
49  GCA GTC TTC GTT TCG GCT AGC TTG TGG GTC ACA GTC TAT TAT GGG GTA
    CGT CAG AAG CAA AGC CGA TCG AAC ACC CAG TGT CAG ATA ATA CCC CAT

+1  P  V  W  K  E  A  T  T  T  L  F  C  A  S  D  A
97  CCT GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT
    GGA CAC ACC TTT CTT CGT TGG TGG TGA GAT AAA ACA CGT AGT CTA CGA

+1  K  A  Y  D  T  E  V  H  N  V  W  A  T  H  A  C
145 AAA GCA TAT GAT ACA GAA GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT
    TTT CGT ATA CTA TGT CTT CAT GTA TTA CAA ACC CGG TGT GTA CGG ACA

+1  V  P  T  D  P  N  P  Q  E  V  V  L  G  N  V  T
193 GTA CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GGA AAT GTG ACA
    CAT GGG TGT CTG GGG TTG GGT GTT CTT CAT CAT AAC CCT TTA CAC TGT

+1  E  N  F  N  M  G  K  N  N  M  V  E  Q  M  H  E
241 GAA AAT TTT AAC ATG GGG AAA AAT AAC ATG GTA GAA CAG ATG CAT GAA
    CTT TTA AAA TTG TAC CCC TTT TTA TTG TAC CAT CTT GTC TAC GTA CTT

+1  D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K  L
289 GAT ATA ATT AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA
    CTA TAT TAA TCA AAT ACC CTA GTT TCG GAT TTC GGT ACA CAT TTT AAT
```

FIG.2B

```
+1   T  P  L  C  V  T  L  N  C  T  K  L  K  N  S  T
337  ACC CCA CTC TGT GTT ACT TTA AAT TGC ACT AAG TTG AAG AAT AGT ACT
     TGG GGT GAG ACA CAA TGA AAT TTA ACG TGA TTC AAC TTC TTA TCA TGA

+1   D  T  N  N  T  R  W  G  T  Q  E  M  K  N  C  S
385  GAT ACC AAT AAT ACT AGA TGG GGA ACA CAA GAA ATG AAA AAC TGC TCT
     CTA TGG TTA TTA TGA TCT ACC CCT TGT GTT CTT TAC TTT TTG ACG AGA

+1   F  N  I  S  T  S  V  R  N  K  M  K  R  E  Y  A
433  TTC AAC ATC AGC ACA AGT GTA AGA AAT AAG ATG AAG AGA GAA TAT GCA
     AAG TTG TAG TCG TGT TCA CAT TCT TTA TTC TAC TTC TCT CTT ATA CGT

+1   L  F  Y  S  L  D  I  V  P  I  D  N  D  N  T  S
481  CTT TTT TAT AGT CTT GAT ATA GTA CCA ATA GAT AAT GAT AAT ACT AGC
     GAA AAA ATA TCA GAA CTA TAT CAT GGT TAT CTA TTA CTA TTA TGA TCG

+1   Y  R  L  R  S  C  N  T  S  I  I  T  Q  A  C  P
529  TAT AGG TTA AGA AGT TGT AAT ACC TCA ATC ATT ACA CAG GCC TGT CCA
     ATA TCC AAT TCT TCA ACA TTA TGG AGT TAG TAA TGT GTC CGG ACA GGT

+1   K  V  S  F  E  P  I  P  I  H  F  C  A  P  A  G
577  AAG GTA TCC TTT GAG CCA ATT CCC ATA CAT TTT TGT GCC CCG GCT GGT
     TTC CAT AGG AAA CTC GGT TAA GGG TAT GTA AAA ACA CGG GGC CGA CCA

+1   F  A  I  L  K  C  N  N  K  T  F  N  G  T  G  P
625  TTT GCG ATT CTA AAG TGT AAC AAT AAA ACG TTC AAT GGA ACA GGA CCA
     AAA CGC TAA GAT TTC ACA TTG TTA TTT TGC AAG TTA CCT TGT CCT GGT
```

FIG.2C

```
+1   C  T  N  V  S  T  V  Q  C  T  H  G  I  R  P  V
673  TGT ACA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA GTA
     ACA TGT TTA CAG TCG TGT CAT GTT ACA TGT GTA CCT TAA TCC GGT CAT

+1   V  S  T  Q  L  L  L  N  G  S  L  A  E  E  E  V
721  GTA TCA ACT CAA CTG CTG TTA AAT GGC AGC CTA GCA GAA GAA GAG GTA
     CAT AGT TGA GTT GAC GAC AAT TTA CCG TCG GAT CGT CTT CTT CTC CAT

+1   V  I  R  S  E  N  F  T  N  N  A  K  T  I  I  V
769  GTA ATT AGA TCT GAA AAT TTC ACA AAC AAT GCT AAA ACC ATA ATA GTA
     CAT TAA TCT AGA CTT TTA AAG TGT TTG TTA CGA TTT TGG TAT TAT CAT

+1   Q  L  N  E  S  V  E  I  N  C  T  R  P  N  N  N
817  CAG CTA AAT GAA TCT GTA GAA ATT AAT TGT ACA AGA CCC AAC AAC AAT
     GTC GAT TTA CTT AGA CAT CTT TAA TTA ACA TGT TCT GGG TTG TTG TTA

+1   T  R  K  S  I  H  I  G  P  G  R  A  F  Y  T  T
865  ACA AGA AAA AGT ATA CAT ATA GGA CCA GGG AGA GCA TTT TAT ACA ACA
     TGT TCT TTT TCA TAT GTA TAT CCT GGT CCC TCT CGT AAA ATA TGT TGT

+1   G  D  I  I  G  D  I  R  Q  A  H  C  N  I  S  R
913  GGA GAT ATA ATA GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT AGA
     CCT CTA TAT TAT CCT CTA TAT TCT GTT CGT GTA ACA TTG TAA TCA TCT

+1   T  N  W  T  N  T  L  K  R  V  A  E  K  L  R  E
961  ACA AAC TGG ACT AAC ACT TTA AAA AGG GTA GCT GAA AAA TTA AGA GAA
     TGT TTG ACC TGA TTG TGA AAT TTT TCC CAT CGA CTT TTT AAT TCT CTT
```

FIG.2D

```
   +1  K  F  N  N  T  T  I  V  F  N  Q  S  S  G  G  G  D
1009  AAA TTT AAT AAT ACA ACA ATA GTC TTT AAT CAA TCC TCA GGA GGG GAC
      TTT AAA TTA TTA TGT TGT TAT CAG AAA TTA GTT AGG AGT CCT CCC CTG

   +1  P  E  I  V  M  H  S  F  N  C  G  G  E  F  F  Y
1057  CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC
      GGT CTT TAA CAT TAC GTG TCA AAA TTA ACA CCT CCC CTT AAA AAG ATG

   +1  C  N  T  T  Q  L  F  N  S  T  W  N  E  T  N  S
1105  TGT AAT ACA ACA CAA CTG TTT AAT AGT ACT TGG AAT GAA ACT AAC AGT
      ACA TTA TGT TGT GTT GAC AAA TTA TCA TGA ACC TTA CTT TGA TTG TCA

   +1  E  G  N  I  T  S  G  T  I  T  L  P  C  R  I  K
1153  GAA GGA AAT ATC ACC AGT GGA ACT ATA ACA CTC CCA TGC AGA ATA AAA
      CTT CCT TTA TAG TGG TCA CCT TGA TAT TGT GAG GGT ACG TCT TAT TTT

   +1  Q  I  I  N  M  W  Q  E  V  G  K  A  M  Y  A  P
1201  CAA ATT ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT GCC CCT
      GTT TAA TAT TTG TAC ACC GTC CTT CAT CCT TTT CGT TAC ATA CGG GGA

   +1  P  I  G  G  Q  I  K  C  L  S  N  I  T  G  L  L
1249  CCC ATC GGA GGA CAA ATT AAA TGT TTG TCA AAC ATC ACA GGG CTG TTA
      GGG TAG CCT CCT GTT TAA TTT ACA AAC AGT TTG TAG TGT CCC GAC AAT

   +1  L  T  R  D  G  G  S  D  N  S  S  S  G  K  E  I
1297  TTA ACA AGA GAT GGT GGT AGT GAT AAC AGT AGT AGT GGG AAA GAG ATC
      AAT TGT TCT CTA CCA CCA TCA CTA TTG TCA TCA TCA CCC TTT CTC TAG
```

FIG.2E

```
+1   F  R  P  G  G  G  D  M  R  D  N  W  R  S  E  L
1345 TTC AGA CCT GGA GGG GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA
     AAG TCT GGA CCT CCC CCT CTA TAC TCC CTG TTA ACC TCT TCA CTT AAT

+1   Y  K  Y  K  V  V  K  I  E  P  L  G  I  A  P  T
1393 TAT AAA TAT AAG GTA GTA AAA ATT GAA CCA TTA GGA ATA GCA CCC ACC
     ATA TTT ATA TTC CAT CAT TTT TAA CTT GGT AAT CCT TAT CGT GGG TGG

+1   K  A  K  R  R  V  V  Q  R  E  K  R  A  V  G  I
1441 AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA AAA AGA GCA GTG GGA ATA
     TTC CGT TTC TCT TCT CAC CAC GTC TCT CTT TTT TCT CGT CAC CCT TAT

+1   G  A  M  F  L  G  F  L  G  A  A  G  S  T  M  G
1489 GGA GCC ATG TTC CTT GGG TTC TTG GGA GCA GCA GGA AGC ACT ATG GGC
     CCT CGG TAC AAG GAA CCC AAG AAC CCT CGT CGT CCT TCG TGA TAC CCG

+1   A  A  S  L  T  L  T  V  Q  A  R  Q  L  L  S  G
1537 GCA GCG TCA CTA ACG CTG ACG GTA CAG GCC AGA CAA TTA TTG TCT GGT
     CGT CGC AGT GAT TGC GAC TGC CAT GTC CGG TCT GTT AAT AAC AGA CCA

+1   I  V  Q  Q  Q  N  N  L  L  R  A  I  E  A  Q  Q
1585 ATA GTG CAG CAG CAA AAC AAT TTG CTG AGG GCT ATT GAG GCG CAA CAG
     TAT CAC GTC GTC GTT TTG TTA AAC GAC TCC CGA TAA CTC CGC GTT GTC

+1   H  L  L  Q  L  T  V  W  G  I  K  Q  L  Q  A  R
1633 CAC CTG TTG CAA CTC ACA GTC TGG GGC ATC AAG CAG CTC CAG GCA AGA
     GTG GAC AAC GTT GAG TGT CAG ACC CCG TAG TTC GTC GAG GTC CGT TCT
```

FIG.2F

```
   +1  V   L   A   L   E   R   Y   L   Q   D   Q   R   F   L   G   M
1681  GTC CTG GCT CTG GAA AGA TAC CTA CAG GAT CAA CGG TTC CTA GGG ATG
      CAG GAC CGA GAC CTT TCT ATG GAT GTC CTA GTT GCC AAG GAT CCC TAC

   +1  W   G   C   S   G   K   L   I   C   T   T   A   V   P   W   N
1729  TGG GGT TGC TCT GGA AAA CTC ATC TGC ACC ACT GCT GTG CCT TGG AAT
      ACC CCA ACG AGA CCT TTT GAG TAG ACG TGG TGA CGA CAC GGA ACC TTA

   +1  A   S   W   S   N   K   N   L   S   Q   I   W   D   N   M   T
1777  GCT AGT TGG AGT AAT AAA AAT CTA AGT CAG ATT TGG GAT AAC ATG ACC
      CGA TCA ACC TCA TTA TTT TTA GAT TCA GTC TAA ACC CTA TTG TAC TGG

   +1  W   M   E   W   E   R   E   I   S   N   Y   T   E   I   I   Y
1825  TGG ATG GAG TGG GAG AGA GAA ATA AGC AAT TAC ACA GAG ATA ATA TAT
      ACC TAC CTC ACC CTC TCT CTT TAT TCG TTA ATG TGT CTC TAT TAT ATA

   +1  S   L   I   E   E   S   Q   N   Q   Q   E   K   N   E   L   D
1873  AGC TTA ATT GAA GAA TCG CAG AAC CAA CAA GAA AAG AAT GAA CTA GAC
      TCG AAT TAA CTT CTT AGC GTC TTG GTT GTT CTT TTC TTA CTT GAT CTG

   +1  L   L   Q   L   D   K   W   A   S   L   W   N   W   F   D   I
1921  TTA TTA CAA TTG GAT AAG TGG GCA AGT TTG TGG AAT TGG TTT GAC ATA
      AAT AAT GTT AAC CTA TTC ACC CGT TCA AAC ACC TTA ACC AAA CTG TAT

   +1  T
1969  ACA
      TGT
```

Note: shown is the translation of the sense strain with it's anti-sense strain below it that was cloned into the pCMV3 vector described in the text.

Effector responses elicited by intramuscular injection of the plasmid, pCMV.gp140.BX08, into BALB/c mice

Immunization:	100.0 ug pCMV.gp.BX08 + 100.0 ug pCMV	100.0 ug pCMV.gp140.BX08 100.0 ug pCMV	100.0 ug pCMV
In vitro re-stimulation:	with autologous CLP-501 pulsed LPS blasts (■)	with autologous CLP-504 pulsed LPS blasts (●)	with autologous CLP-501 pulsed LPS blasts (▲)
	with autologous LPS Blasts (▲)		with autologous CLP-504 pulsed LPS blasts (●)
Target:	CLP-501-pulsed P815	CLP-504-pulsed P815	CLP-501-pulsed P815 for CLP-501-pulsed blast re-stimulation CLP-504-pulsed P815 for CLP-504-pulsed blast re-stimulation

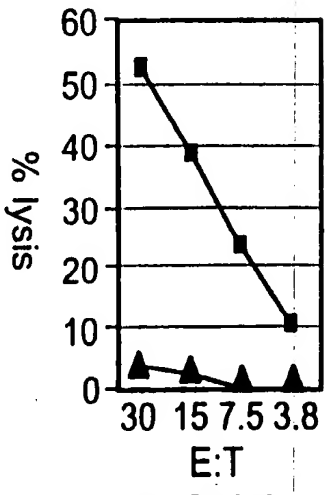

FIG.3A

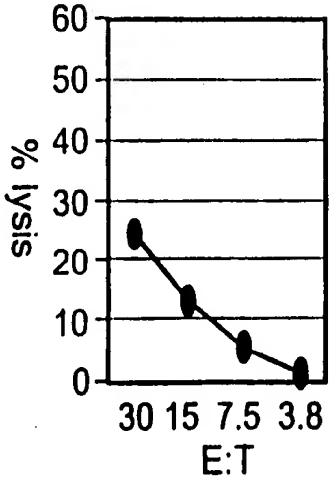

FIG.3B

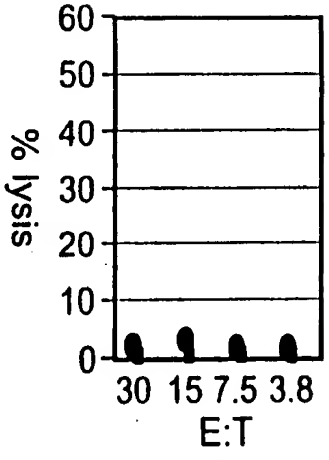

FIG.3C

Effector responses elicited by gene gun delivery of the plasmid, pCMV.gp140.BX08, into BALB/c mice

Immunization:	0.7 ug pCMV.gp140.BX08 + 0.7 ug pCMV	0.7 ug pCMV.gp140.BX08 + 0.7 ug pCMV	1.4 ug pCMV
In vitro re-stimulation:	with autologous CLP-501 pulsed LPS blasts (■) with autologous LPS Blasts (▲)	with autologous CLP-504 pulsed LPS blasts (●)	with autologous CLP-501 pulsed LPS blasts (■) with autologous CLP-504 pulsed LPS blasts (●)
Target:	CLP-501-pulsed P815	CLP-504-pulsed P815	CLP-501-pulsed P815 for CLP-501-pulsed blast re-stimulation CLP-504-pulsed P815 for CLP-504-pulsed blast re-stimulation

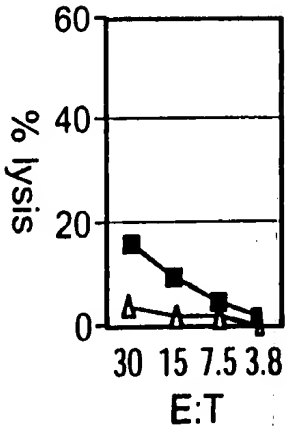

FIG.4A

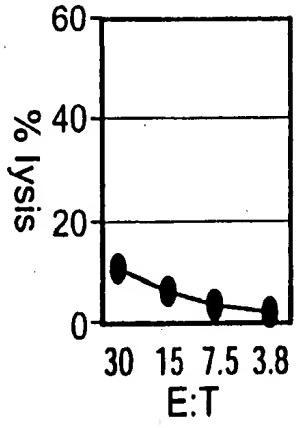

FIG.4B

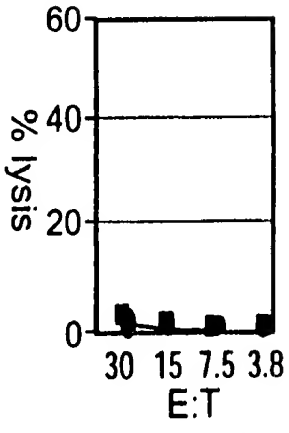

FIG.4C

Sequence of Bam HI fragment containing HIV-1 gp 140 sequences for alphavirus expression vectors.

gatccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtggagcagtcttcgtttcggctagcttgtgg
gtcacagtctattatggggtacctgtgtggaaagaagcaaccaccactctattttgtgcatcagatgctaaagcatatgatac
agaagtacataatgtttgggccacacatgcctgtgtacccacagaccccaacccacaagaagtagtattgggaaatgtga
cagaaaatttaacatggggaaaaataacatggtagaacagatgcatgaagatataattagtttatgggatcaaagccta
aagccatgtgtaaaattaaccccactctgtgttactttaaattgcactaagttgaagaatagtactgataccaataatactaga
tggggaacacaagaaatgaaaaactgctctttcaacatcagcacaagtgtaagaaataagatgaagagagaatatgca
ctttttatagtcttgatatagtaccaatagataatgataatactagctataggttaagaagttgtaatacctcaatcattacaca
ggcctgtccaaaggtatcctttgagccaattcccatacatttttgtgccccggctggttttgcgattctaaagtgtaacaataaa
acgttcaatggaacaggaccatgtacaaatgtcagcacagtacaatgtacacatggaattaggccagtagtatcaactca
actgctgttaaatggcagcctagcagaagaagaggtagtaattagatctgaaaatttcacaaacaatgctaaaaccataa
tagtacagctaaatgaatctgtagaaattaattgtacaagacccaacaacaatacaagaaaaagtatacatataggacc
agggagagcattttatacaacaggagatataataggagatataagacaagcacattgtaacattagtagaacaaactgg
actaacacttaaaaagggtagctgaaaaattaagagaaaaatttaataatacaacaatagtctttaatcaatcctcagga
ggggacccagaaattgtaatgcacagttttaattgtggaggggaatttttctactgtaatacaacacaactgtttaatagtactt
ggaatgaaactaacagtgaaggaaatatcaccagtggaactataacactcccatgcagaataaaacaaattataaacat
gtggcaggaagtaggaaaagcaatgtatgcccctcccatcggaggacaaattaaatgtttgtcaaacatcacagggctgt
tattaacaagagatggtggtagtgataacagtagtagtgggaaagagatcttcagacctggaggggagatatgaggga
caattggagaagtgaattatataaatataaggtagtaaaaattgaaccattaggaatagcacccaccaaggcaaagaga
agagtggtgcagagagaaaaaagagcagtgggaataggagccatgttccttgggttcttgggagcagcaggaagcact
atgggcgcagcgtcactaacgctgacggtacaggccagacaattattgtctggtatagtgcagcagcaaaacaatttgct
gagggctattgaggcgcaacagcatcctgttgcaactcacagtctggggcatcaagcagctccaggcaagagtcctggct
ctggaaagatacctacaggatcaacggttcctagggatgtggggttgctctggaaaactcatctgcaccactgctgtgcctt
ggaatgctagttggagtaataaaaatctaagtcagatttgggataacatgacctggatggagtgggagagagaaataag
caattacacagagataatatatagcttaattgaagaatcgcagaaccaacaagaaaagaatgaactagacttattacaat
tggataagtgggcaagtttgtggaattggtttgacataacataag

FIG.5

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/CA 00/00190

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/49 C07K14/16 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LU S ET AL: "Immunogenicity of DNA vaccines expressing human immunodeficiency virus type 1 envelope glycoprotein with and without deletions in the V1/2 and V3 regions." AIDS RESEARCH AND HUMAN RETROVIRUSES, JAN 20 1998, 14 (2) P151-5, XP000907375 UNITED STATES	1,3,6,8, 11,15
Y	abstract; figure 1	2,4,7,9
X	WO 93 18055 A (US HEALTH) 16 September 1993 (1993-09-16) page 7, line 16; claims 1,5,12,28	16,17
X	WO 97 31115 A (DAVIES MARY ELLEN ;PERRY HELEN C (US); SHIVER JOHN W (US); FREED D) 28 August 1997 (1997-08-28)	1,3,15
A	claims 1-10; figure 1; example 7	6,8,11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2000	14/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 00/00190

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	YAHI N ET AL: "STRUCTURAL VARIABILITY OF ENV AND GAG GENE PRODUCTS FROM A HIGHLY CYTOPATHIC STRAIN OF HIV-1" ARCHIVES OF VIROLOGY 1992, vol. 125, no. 1-4, 1992, pages 287-298, XP000907410 ISSN: 0304-8608	1,3,15
A	the whole document	6,8,11
Y	VERRIER FLORENCE C ET AL: "Antibodies to several conformation-dependent epitopes of gp120/gp41 inhibit CCR-5-dependent cell-to-cell fusion mediated by the native envelope glycoprotein of a primary macrophage-tropic HIV-1 isolate." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1997, vol. 94, no. 17, 1997, pages 9326-9331, XP002138893 ISSN: 0027-8424 abstract; figure 1	2,4,7,9
A	WO 94 28929 A (GENENTECH INC ;BERMAN PHILLIP W (US); NAKAMURA GERALD R (US)) 22 December 1994 (1994-12-22) page 32 -page 35; claims 1-33	1-7,15, 19
A	O'BRIEN W A ET AL: "HIV-1 TROPISM FOR MONONUCLEAR PHAGOCYTES CAN BE DETERMINED BY REGIONS OF GP120 OUTSIDE THE CD4-BINDING DOMAIN" NATURE (LONDON) 1990, vol. 348, no. 6296, 1990, pages 69-73, XP002138883 ISSN: 0028-0836 the whole document	1-7,15, 19
A	-& EMBL DATABASE ; ACCESSION NUMBER U63632,30-August 1996-(1996-08-30), XP002138884 figure GP160	1-7,15, 19

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INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/CA 00/00190

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WESTERVELT P ET AL: "IDENTIFICATION OF A DETERMINANT WITHIN THE HUMAN IMMUNODEFICIENCY VIRUS 1 SURFACE ENVELOPE GLYCOPROTEIN CRITICAL FOR PRODUCTIVE INFECTION OF PRIMARY MONOCYTES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1991, vol. 88, no. 8, 1991, pages 3097-3101, XP002138885 ISSN: 0027-8424 the whole document	1-7,15, 19
A	-& EMBL DATABASE ; ACCESION NUMBER M60472, 1 May 1991 (1991-05-01), XP002138886 the whole document	1-7,15, 19

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INTERNATIONAL SEARCH REPORT

International Application No. PCT/CA 00 00190

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. nal Application No
PCT/CA 00/00190

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		AU 7047894 A	03-01-1995
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